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Evaluation of Anti-inflammatory activity of stems of *Passiflora foetida* Linn. in rats

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ABSTRACT

Effects of ethanolic extract of the stems of *Passiflora foetida was* studied in albino rats to assess its anti-inflammatory property against the paw edema and cotton pellet granuloma. The study revealed that the fruits extract at the dose levels of 100, 200 and 400mg/kg body weight significantly possesses its anti-inflammatory activity by acute and sub acute models.

Keywords: *Passiflora foetida,* Anti inflammatory activity, Cotton pellet granuloma, Paw edema, carrageenan.

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INTRODUCTION

Plants continue to be major resources for therapeutic compounds. Plants have always been an exemplary source of drugs and many of the currently available drugs have been derived directly or indirectly from them [1]. Ethnobotanical and ubiquitous plants serve as a rich resource of natural drugs for research and development. Medicinal plant-based drugs owe the advantage of being simple, effective and exhibit broad spectrum activity. Medicinal plant products when compared to their synthetic counterparts minimize the adverse effects [2].As a result, a search for other alternatives seems necessary and beneficial. Medicinal plants having a wide variety of chemicals from which novel anti-inflammatory agents could be discovered. Scientific studies are required to judge their efficacy. *Passiflora foetida* (Linn) belongs to Passifloraceae family is a herbaceous climber, native of Tropical America and found wild in several parts of India[3]. It is commonly called as Stinking passion flower [4-6]. The whole plant is used in the treatment of insomnia and anxiety [7].Present study was carried out to assess the anti inflammatory activity of stem extract of *Passiflora foetida* .

MATERIAL AND METHODS

Plant Material and Preparation Of Ethanolic Extract

The stems of *Passiflora foetida* were collected from the local areas of Mangalore, Karnataka. The plant of *Passiflora foetida* had been identified and authenticated by the botanist Dr Noeline Pinto. Professor & Head of Botany Department, St. Agnes College, Mangalore. A voucher specimen is was deposited in NGSM Institute of Pharmaceutical Sciences,Mangalore. The stems were cleaned, dried in shade and broken down into small pieces and powdered into a coarse powder by a mechanical grinder. The powder was then passed through sieve no.40 and extracted with ethanol(95%) in soxhlet extractor exhaustively for 20-24 hours. The extract was concentrated to dryness under reduced pressure and controlled temperature using flash evaporator.Preliminary phytochemical analysis was performed for testing the different chemical groups present in ethanolic extract by standard procedure.[8]

Animals and Housing Condition

Adult albino rats of either sex weighing between 180- 200 g were selected for the antiinflammatory study and maintained under standard laboratory conditions (temperature 25 ± 2 C with dark and light cycle 12/12 h).They were allowed free access to standard dry pellet diet (Hindustan Lever, Mumbai,India) and water *ad libitum*. The animals were fasted for 10 h with water *ad libitum* before commencement of treatments. All the experiments were performed within the guidelines of the Institutional ethical committee of KSHEMA.Deralakatte, Mangalore. (KSHEMA /IAEC / 13/2011)

Acute Toxicity Studies

Acute toxicity study was conducted to determine the median lethal dose (LD50) of the ethanolic extract of stems of *Passiflora foetida L*. The acute toxicity study was carried out in adult female albino rats by "up and down"[9] method and OECD guidelines 425 [10]**.** The animals were fasted overnight and divided into various groups.Then they were treated with a oral dose ranging from of 100mg/kg to 2000mg/Kg of body weight with the ethanolic extract of the stems of *Passiflora foetida L* suspended in 0.5% NaCMC. The animals were observed continuously for 2-3 hours for general behavioral, neurological, autonomic profiles and finally death after 24 hours. There was no mortality and no signs of toxicity upto the dose of 2000 mg/kg bodyweight of ethanolic extract of stems of *Passiflora foetida L* and and the extract were found to be safe at this dose level

Carrageenan-induced Acute Paw Edema in Rats [11-13]

Preparation of Carrageenan Suspension

1% suspension of carrageenan was prepared by sprinkling 100 mg of carrageenan powder in 10 ml of saline (0.9% w/v NaCl) solution and set aside to soak for 1 h. A homogenous suspension was then obtained by thorough mixing with magnetic stirrer.

Equipment: Plethysmograph (Mercury Displacement)

Procedure

A mark was made on both the hind paws just below the tibio-tarsal junction so that every time the paw could be dipped in the mercury column of plethysmograph up to the mark to ensure constant paw volume. After 30 min of above treatment, an inflammatory edema was induced in the left hind paw by injecting 0.1 ml carrageenan (1%) in the planter tissue hind paw of all the animals. The right paw served as a reference to non-inflamed paw for comparison. The initial paw volume was measured plethysmographically within 30 sec of the injection. The relative increase in the paw volume was measured in control, standard and treated groups upto 3h after carrageenan injection. The percent increase in paw volume over the initial reading was calculated. This increase in the paw volume in animals treated with standard drug and the different doses of ethanolic extract of the stems of *Passiflora foetida L* were compared with the increase in paw volume of untreated control animals after 3 h. The percentage inhibition of edema volume was calculated using the formula,

$$\% \text{ Inhibition} = [Vt/Vc - 1] \times 100$$

Where, Vt and Vc are the relative changes in the edema of the test and control respectively. The results were expressed as % inhibition of edema over the untreated control group.

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Cotton Pellet Granuloma Method (Sub-Acute Inflammatory Model) [14-16]

Procedure

Method has been described first by Meier et al (1950) who showed that foreign body granulomas were provoked in rats by subcutaneous implantation of pellets of cotton. After several days histologically giant cells and undifferentiated connective tissue can be observed besides the fluid infiltration. The amount of newly formed connective tissue can be measured by weighing the dried pellets after removal.

Male albino rats with an average weight of 200 g were selected for the study. They were divided into 5 different groups, consisting six each in a group. Weighed and numbered the rats. The first group served as control. Second group served as standard drug treated. Third, fourth and fifth group 100, 200,400 mg/kg body weight (p.o.) ethanolic extract of stems of *Passiflora Foetida* (suspended in 0.5% NaCMC).The rats anaesthetized using anesthetic ether. The dorsal skin shaved and disinfected with 70% ethanol. An incision was made in the lumbar region. By a blunted forceps subcutaneous tunnels were formed and presterillized cotton pellets weighing about 20 mg± 0.05 (the cotton pellets were sterilized in an autoclave for 30-45 min under 15 lb pressures) was placed on both axillae and groin regions. The animals were treated for 0-9 days with standard and test drugs to the different group respectively.

On the 10th day the animals were anaesthetized with ether and pellets were removed, cleaned and dried at 60-70 0C for 6 h. Granuloma weight was obtained by deducting the weight of the cotton pellets on 0 day (i.e., before start of the experiment) from the weight of the cotton pellet on 9th day (i.e., at the end of the experiment) the average weight of the pellets of the control group as well as the test group were calculated.The percent change of the granuloma weight relative to vehicle control group was determined by using the formula,

$$\textbf{Percentage inhibition} = [1 - Gt/Gc] \times 100$$

Where, Gt = Mean dry weight of granulation tissue in treated groups.
Gc = Mean dry weight of granulation tissue in control group.

Statistical Analysis

All values are expressed as mean + SEM. One way ANOVA followed by post hoc test (Dunnet t-Test)

RESULTS AND DISCUSSION

Table 1 shows the effect of drug and extracts treatment on carrageenan induced oedema. Edema effect of 400 mg/kg dose of alcoholic extract of *Passiflora foetida* which was nearer to that of 100 mg/kg body weight of Diclofenac sodium. The edema suppressant effect was significant (at $P<0.05$) in all doses levels when compared to control, though the alcoholic

stem extract of *Passiflora foetida* showed dose response inhibition of inflammation. Table 2 shows the effect of drug treatment on the mean weights of cotton pellet. The alcoholic fruits extract of at dose levels (100, 200 and 400 mg/kg) inhibited the granuloma tissue formation. Also showed significant dose proportionate inhibited effect on the granuloma weight. The inhibitory effect of the alcoholic extract of at the dose of 400 mg/kg body weight was found to be almost nearer to that of 100 mg/kg of Diclofenac.

Table 1: Effect of alcoholic stem extract of *Passiflora foetida* on Carrageenan induced paw edema

Treatment	Dose (mg/kg)	Increase in paw volume (min)/ % Inhibition of edema volume			
		0h	1h	2h	3h
Control	5 ml/kg	0.073±0.05	0.093±0.016	0.160±0.006	0.221±0.008
Diclofenac sodium	100	0.025±0.009** 65.75%	0.023±0.004** 75.28%	0.033±0.05** 79.37%	0.043±0.005** 80.54%
Ethanolic stem extract of *Passiflora foetida*	100	0.062±0.004** 15.06%	0.085±0.007** 8.06%	0.118±0.006** 26.025	0.146±0.008** 33.93
	200	0.055±0.005** 24.65%	0.056±0.006** 39.78%	0.075±0.04** 53.12%	0.08±0.007** 61.99%
	400	0.040±0.01** 45.20%	0.041±0.008** 55.91%	0.070±0.005** 56.25%	0.051±0.010** 76.956%

***The mean difference is significant at the 0.05 level, when compared to the control group.*

Table 2: Effect of alcoholic Stem extract of *Passiflora foetida* on the weight of cotton pellet Granuloma

Treatment	Dose (mg/kg)	Granulation weight in mg	% inhibition
Control	5ml/kg	76.03±1.89**	-
Diclofenac	100	34.00±2.31**	55%
Ethanolic extract of stems of *Passiflora foetida*	100	50.83±0.89**	33%
	200	43.43±1.57**	43%
	400	38.10±3.07**	50%

***The mean difference is significant at the 0.05, when compared to the control group*

Carrageenan induced acute inflammation in animals is one of the most suitable test procedures to screen anti-inflammatory agents. The carrageenan induced edema is mediated by activation of platelet activating factor (PAF), prostaglandins and other inflammatory mediators [17]. The first phase is attributed to the release of histamine 5-HT and kinins. The second phase is related to the release of prostaglandins.Carrageenan also induces a protein rich exudate containing large number of neutrophills [18].

In cotton pellet granuloma model, inflammation and granuloma develops during a period of several days. This model is the indication for the proliferative phase of inflammation. Inflammation involves proliferation of macrophages, neutrophils and fibroblasts, which are

basic sources for granuloma formation. So, the decrease in granuloma weight indicates the suppression of proliferation phase which was effectively inhibited by alcoholic stem extract of *Passiflora foetida* as indicated by our study.

CONCLUSION

In the present work, medicinally useful plant in the Indian system of medicine,stems of *Passiflora foetida* Linn., were selected. Acute toxicity studies of the extracts revealed that the drug was non toxic upto the dose level of 2000 mg/kg body weight. Ethanolic extract of stems of *Passiflora foetida* at the dose of 400mg/kg has significant effect on anti-inflammatory activity.

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